Acta Medica Okayama

Volume 49, *Issue* 1 1995 *Article* 5

FEBRUARY 1995

Major Basic Protein, Eosinophil Cationic Protein, and Arylsulfatase in Nasal Secretions of Patients with Japanese Cedar Pollinosis

Keiko Nishioka[*] Teruhiro Ogawa[†] Chisato Saito[‡]

Satoko Nishioka[**] Fumio Nakagawa[††]

Takuya Ohomichi[‡‡] Yu Masuda[§]

[*]Hiroshima City Hospital,

[†]Hiroshima City Hospital,

[‡]Okayama City Hospital,

[**]Okayama University Medical School,

[††]Okayama University Medical School,

[‡‡]Okayama University Medical School,

[§]Okayama University Medical School,

Major Basic Protein, Eosinophil Cationic Protein, and Arylsulfatase in Nasal Secretions of Patients with Japanese Cedar Pollinosis*

Keiko Nishioka, Teruhiro Ogawa, Chisato Saito, Satoko Nishioka, Fumio Nakagawa, Takuya Ohomichi, and Yu Masuda

Abstract

In 15 patients with Japanese cedar pollinosis and 10 healthy control subjects, levels of major basic protein (MBP), eosinophil cationic protein (ECP), and arylsulfatase B (As) in the nasal secretions were examined before and after challenge with Japanese cedar pollen extract. The MBP and ECP levels in the patients were significantly higher 30 min after challenge than those before challenge ($P < 0.005$). MBP and ECP levels after challenge were significantly higher in the nasal secretions of patients than in the controls (MBP: $P < 0.01$, ECP: $P < 0.05$). The level of As after challenge was significantly higher in the nasal secretions of patients than in the controls. These results suggest that eosinophils activate or modify the immediate, nasal allergic reaction and have a role in regulating immunological responses.

KEYWORDS: major basic protein, eosinophil cationic protein, arylsulphatase B, pollinosis, allergic rhinitis

---

*PMID: 7762407 [PubMed - indexed for MEDLINE]

ACTA MED OOKAYAMA 1995; 49 (1): 29 33

Major Basic Protein, Eosinophil Cationic Protein, and Arylsulfatase in Nasal Secretions of Patients with Japanese Cedar Pollinosis†

Keiko NISHIOKA, Teruhiro OGAWA, Chisato SAITO[a], Satoko NISHIOKA[b], Fumio NAKAGAWA[b], Takuya OHOMICHI[b] and Yu MASUDA[b]

Department of Otolaryngology, Hiroshima City Hospital, Hiroshima 730, [a]Department of Otolaryngology, Okayama City Hospital, Okayama 700 and [b]Department of Otolaryngology, Okayama University Medical School, Okayama 700, Japan

In 15 patients with Japanese cedar pollinosis and 10 helthy control subjects, levels of major basic protein (MBP), eosinophil cationic protein (ECP), and arylsulfatase B (As) in the nasal secretions were examined before and after challenge with Japanese cedar pollen extract. The MBP and ECP levels in the patients were significantly higher 30 min after challenge than those before challenge ($P < 0.005$). MBP and ECP levels after challenge were significantly higher in the nasal secretions of patients than in the controls (MBP: $P < 0.01$, ECP: $P < 0.05$). The level of As after challenge was significantly higher in the nasal secretions of patients than in the controls. These results suggest that eosinophils activate or modify the immediate, nasal allergic reaction and have a role in regulating immunological responses.

Key words: major basic protein, eosinophil cationic protein, arylsulphatase B, pollinosis, allergic rhinitis

Eosinophils are considered to play a role in the allergic reaction in patients with allergic rhinitis. The migration of eosinophils to the site of allergic reaction on the nasal mucosa is induced by chemotactic substances such as eosinophil chemotactic factor of anaphylaxis (ECF-A), leukotriene B_4, platelet-activating factor (PAF), and histamine. The mechanism of action of eosinophil is gradually being elucidated. Specific granules of eosinophils contain eosinophil cationic protein (ECP), major basic protein (MBP), eosinophil peroxidase (EPO), and eosinophil-derived neurotoxin (EDN), and small granules contain arylsulfatase B (As). These factors are closely related to the pathophysiology of diseases that involve histotoxic or allergic reactions.

We previously evaluated the level of ECP in nasal lavage fluids obtained from patients with mite-induced allergic rhinitis before and after provocation testing, and found that the levels had increased significantly 30 min after provocation (1). We applied these methods to our current study. During the pollen season (March–May) when cedar pollinosis is prevalent in Japan, we measured levels of MBP, ECP, and As in the nasal lavage fluids of patients with this condition. Finally we examined the correlation between the levels of MBP and ECP.

Subjects and Methods

We studied 15 patients aged 13 to 43 years (average 28.4 years) with a confirmed diagnosis of Japanese cedar pollinosis. All had typical symptoms of nasal allergy such as sneezing, nasal obstruction, and rhinorrhea during the pollen season. They responded positively to skin tests and to the nasal provocation test with Japanese cedar pollen extract (Torii & Co., Ltd. Tokyo, Japan). In most cases the level of specific immunoglobulin E (IgE) level against the pollen exceeded a score of 2 [0.70 peripheral resistance units (PRU)/ml]. In addition, nearly all of these patients had high levels of eosinophils in their nasal smears. The serum IgE of these patients ranged from 33.7 to 692.0 IU/ml (average 270.9 IU/ml). Before the nasal provocation testing, none of the patients had any symptom such as sneezing or hydrorrhea except for nasal obstruction. The control group consisted of 10 healthy

* To whom correspondence should be addressed.

† This study was presented in part at the 15th World Congress of Otorhinolaryngology Head and Neck Surgery, Istanbul, Turkey, June 1993.

volunteers with no personal or family history of allergic disease, and who responded negatively to skin testing for Japanese cedar pollen extract and other allergens.

Method of nasal washing and nasal provocation test. In accordance with the method of obtaining nasal lavage fluid described in a previous report (1), the nasal cavity of each subject was lavaged twice continuously with 50 ml of physiological saline using a spray washing tools prior to performing provocation testing (first and second washings). After lavage, a circular filter paper about 3 mm in diameter containing extracts of Japanese cedar pollen with a total nitrogen content of 132 μg/ml and a protein nitrogen content of 21.8 μg/ml were placed bilaterally on each inferior turbinate. After 30 min, the nasal cavity was again lavaged with 50 ml of physiological saline (third washing). Each washing was centrifuged at 1,000 rpm for 10 min to separate the cells. The supernatant was immediately stored at -70°C and subsequently examined for levels of MBP, ECP, and As.

MBP assay. The level of MBP was measured by an immunoradiometric assay in accordance with the method of Wagner *et al.* (2) as shown in Fig. 1.

ECP assay. The levels of ECP were measured by radioimmunoassay (RIA) using a kit (Pharmacia Uppsala, Sweden) in accordance with a previous report (1). A standard curve was made before measuring each ECP level, and unknown levels of ECP were read from this curve of radioactivity counts.

Measurement of As level. To determine the levels of As, samples were allowed to react with para-introcatechol as a base, and after a color reaction had occurred, the enzyme activity was measured colorimetrically with a spectrophotometer as shown in Fig. 2.

Statistical analysis. All values are given as

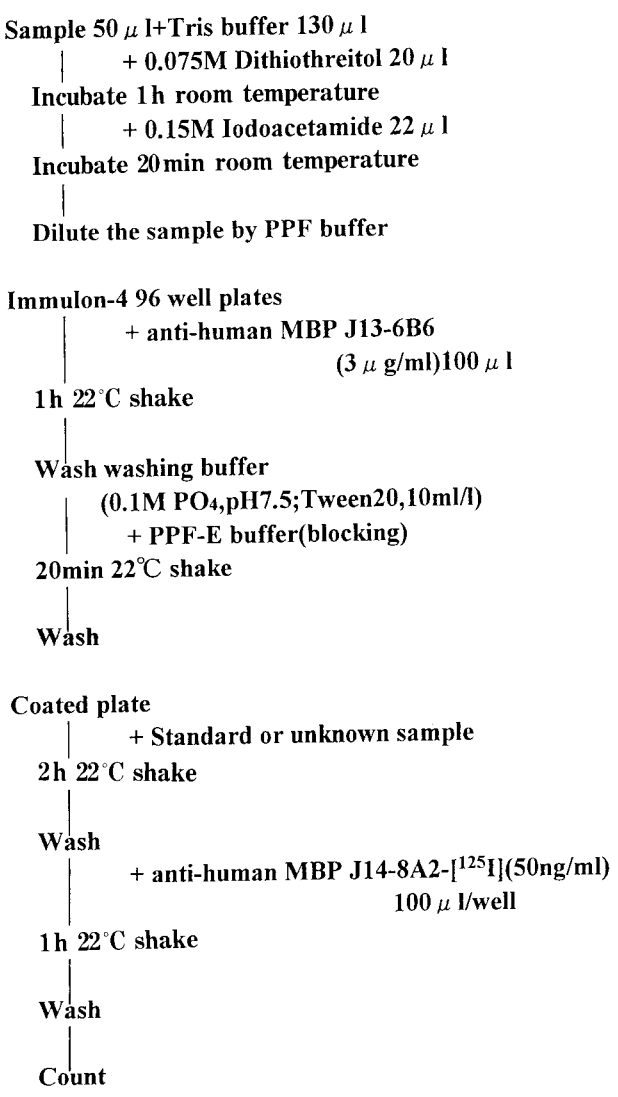

Fig. 1 Method of measuring levels of major basic protein (MBP) by radioimmunoassay (RIA).

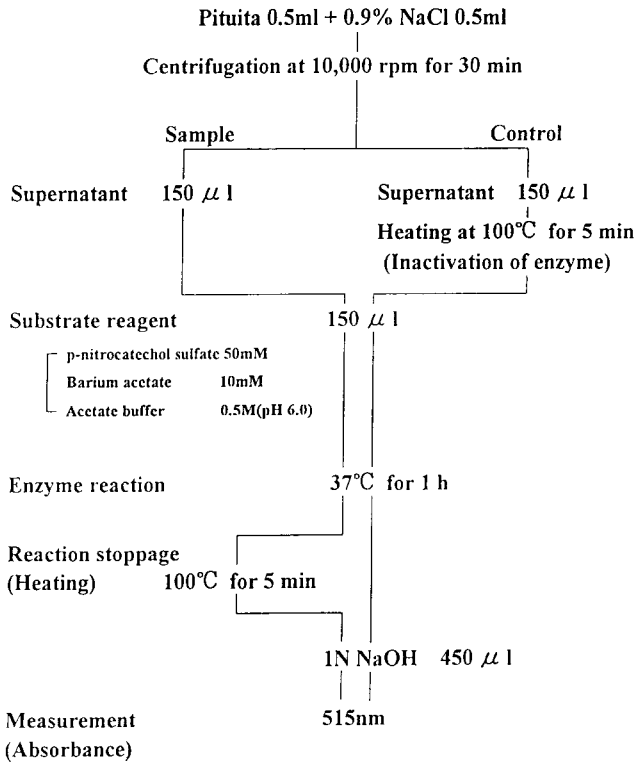

Fig. 2 Method of measuring levels of arylsulfatase.

the mean ± SE and Wilcoxon test was used.

Results

MBP levels in nasal lavage before and after nasal challenge

The MBP level in the nasal lavage fluid of the 15 pollinosis patients was 12.0 ± 7.6 ng/ml in the first washing, 4.6 ± 0.2 ng/ml in the second washing, and 57.1 ± 31.9 ng/ml in the lavage fluid 30 min after challenge. The MBP level in the control group was 6.1 ± 1.7 ng/ml in the first washing, 5.3 ± 0.9 ng/ml in the second washing, and 5.4 ± 1.0 ng/ml 30 min after challenge. The MBP level of patients was significantly higher in the lavage fluid after challenge than in the first and second washings ($P < 0.005$) and significantly exceeded that of the control group ($P < 0.01$) (Fig. 3).

ECP levels in nasal lavage before and after nasal challenge

Fig. 4 shows the level of ECP in the nasal lavage fluid of patients before and after nasal provocation testing with an extract of Japanese cedar pollen. The ECP concentration in the first nasal washing of the pollinosis patients was 5.2 ± 1.9 ng/ml, and 2.7 ± 0.5 ng/ml in the second washing, (before challenge), whereas it was 32.1 ± 12.7 ng/ml (mean ± SE) 30 min after challenge. In the pollinosis patients, the ECP level in the nasal lavage fluid 30 min after challenge was significantly higher than the levels in the first and second washings ($P < 0.005$). In comparison, in the healthy control group, the ECP concentration was negligible both before and after challenge. The ECP level of the patients was significantly higher than that of the control subjects after challenge ($P < 0.05$).

As levels in nasal lavage before and after nasal challenge

The level of As in the nasal lavage fluid of pollinosis patients was 3.8 ± 0.3 U/ml in the first washing, 4.0 ± 0.2 U/ml in the second washing, and 4.6 ± 0.4 U/ml in the lavage fluid after challenge. The As level in the lavage fluid of pollinosis patients after challenge was significantly

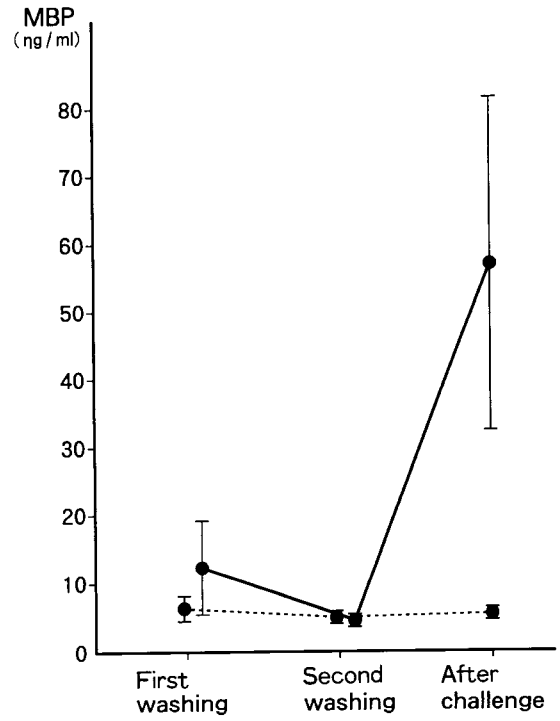

Fig. 3 Amount of major basic protein (MBP) in nasal lavage fluid before and after provocation tests in the patients and the healthy controls. All values are expressed as the mean ± SE.
(—): Patients; (···): Controls.

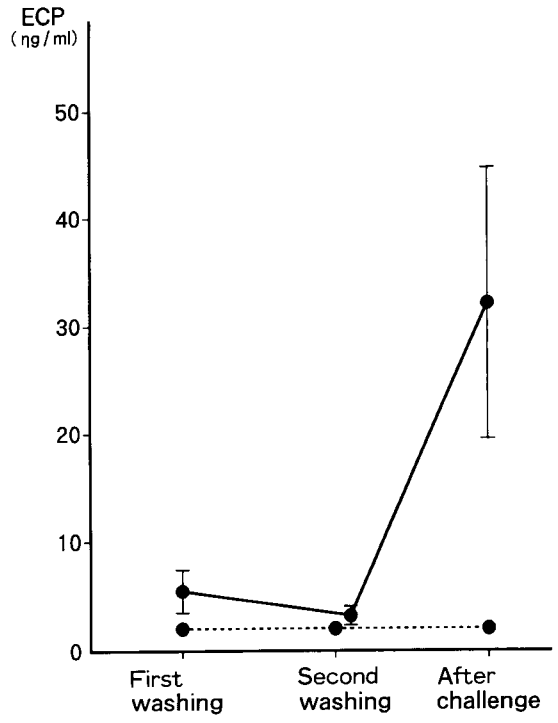

Fig. 4 Amount of eosinophil cationic protein (ECP) in nasal lavage fluid before and after provocation tests in the patients and the healthy controls. All values are expressed as the mean ± SE.
(—): Patients; (···): Controls.

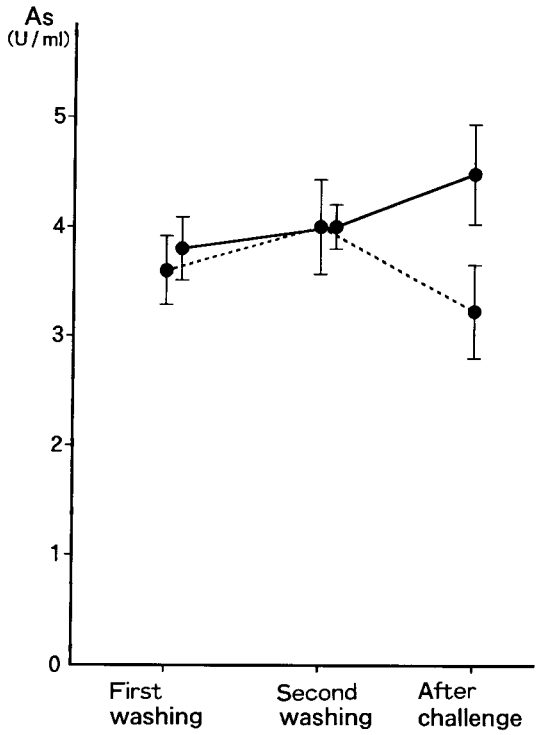

Fig. 5 Amount of arylsulfatase B (As) in nasal lavage fluid before and after provocation tests in the patients and the healthy controls. All values are expressed as the mean ± SE.
(—): Patients; (---): Control.

higher than that of the control group ($P < 0.05$) (Fig. 5).

Correlation between levels of MBP and ECP

The correlation between the levels of MBP and ECP was not so close statistically ($Y = 13.67 + 1.36X$; $r = 0.54$).

Discussion

The ECP present in specific granules of the eosinophils increases during the late asthmatic reaction and is associated with the presence of certain pathologies in such patients (3). ECP and MBP were reported to produce toxic injury to the bronchial mucosa of guinea pigs (4, 5). Since this late reaction occurs 4 to 48h after nasal challenge in 50 % of the pollinosis patients (6), attention has been directed to this reaction as well as to the immediate type 1 allergic reaction. Levels of ECP and MBP have been shown to increase significantly in the nasal lavage fluid of patients with pollinosis during the late phase allergic reaction (7, 8).

The present study revealed that levels of MBP, ECP, and As significantly increased in the nasal lavage fluid of pollinosis patients during the immediate reaction that occurs within 30 min of challenge with Japanese cedar pollen extract. Bascom *et al.* (9) described a significant increase in MBP shortly after nasal challenge with allergen. In our study, the MBP level was much higher than the MBP level reported by Bascom. In our method by nasal washing, all amount of levels of MBP in the nasal cavity of patients could be measured as a results of the immediate allergic reaction in the nasal cavity. These findings suggest that MBP, ECP or As has some role that mediate the late allergic reaction in patients following the immediate allergic reaction.

Although we did not take nonspecific increases in the nasal secretions of the allergic subjects into account as a source of the positive findings, other substances or physiological actions in addition to allergens may increase the levels of MBP and ECP. Most of the MBP, ECP and As may originate in the eosinophils. Whether they arise from eosinophils in the mucous membrane or in the mucous blanket that covers mucous membrane is not yet known.

In our study, the correlation between the level of MBP and ECP was not particularly strong, although this may, in part, be due to the wide variation in the levels in MBP and ECP observed. Studies with a larger population are needed to establish the relation between MBP and ECP.

ECP reportedly stimulates the mast cells of the rat to release histamine (10) and inhibits the proliferation of lymphocytes (11). MBP potentially induces the release of histamine from human basophils and rat mast cells (10). As is thought to inactivate slow reacting substance of anaphylaxis (SRS-A) (12), although these results are controversial. Tanizaki *et al.* reported that the activity of As significantly increased in patients with atopic asthma, who showed a higher eosinophil count (13). It was considered that ECP, MBP, and As could act as a trigger in the late phase reaction several hours later.

References

1. Nishioka K, Saito C, Nagano T, Okano M, Masuda Y and Kuriyama T: Eosinophil cationic protein in the nasal secretions of patients with mite allergic rhinitis. Laryngoscope (1993) **103**, 189-192.
2. Wagner JM, Bartemes K, Vernof K, Dunnette S, Offord KP, Checkel JL,

and Gleich GJ: Analysis of pregnancy-associated major basic protein levels throughout gestation. Placenta (1993) **14**, 671-681.
3. De Monchy JGR, Kauffman HF, Venge P, Koetör GH, Jansen HM, Sluiter HJ and Vries KD: Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. Am Rev Respir Dis (1985) **131**, 373-376.
4. Motojima S, Frigas E, Loegering DA, and Gleich GJ: Toxicity of eosinophil cationic proteins for guinea pig tracheal epithelium *in vitro*. Am Rev Respir Dis (1989) **139**, 801-805.
5. Gleich GJ: The eosinophil and bronchial asthma: Current understanding. J Allergy Clin Immunol (1990) **85**, 422-436.
6. Dvoracek JE, Yunginger JW, Kern EB, Hyatt RE and Gleich GJ: Induction of nasal late-phase reactions by insufflation of ragweed-pollen extract. J Allergy Clin Immunol (1984) **73**, 363-368.
7. Svensson C, Andersson M, Persson CGA, Venge P, Alkner U and Pipkorn U: Albumin, bradykinins, and eosinophil cationic protein on the nasal mucosal surface in patients with hay fever during natural allergen exposure. J Allergy Clin Immunol (1990) **85**, 828-833.
8. Bisgaard H, Gronbørg H, Mygind N, Dahl R, Lindqvist N and Venge P: Allergen-induced increase of eosinophil cationic protein in nasal lavage fluid: Effect of the glucocorticoid budesonide. J Allergy Clin Immunol (1990) **85**, 891-895.
9. Bascom R, Pipkorn U, Proud D, Dunnette S, Gleich G, Lichtenstein LM and Naclerio RM: Major basic protein and eosinophil-derived neurotoxin concentrations in nasal-lavage fluid after antigen challenge: Effect of systemic corticosteroids and relationship to eosinophil influx. J Allergy Clin Immunol (1989) **84**, 338-346.
10. Zheutlin LM, Ackerman SJ, Gleich GJ and Thomas LL: Stimulation of basophil and rat mast cell histamine release by eosinophil granule-derived cationic proteins. J Immunol (1984) **133**, 2180-2185.
11. Peterson CGB, Skoog V and Venge P: Human eosinophil cationic proteins (ECP and EPX) and their suppressive effects on lymphocyte proliferation. Immunobiology (1986) **171**, 1-13.
12. Orange RP, Robert C, Murphy RC and Austen KF: Inactivation of slow reacting substance of anaphylaxis (SRS-A) by arylsulfatases. J Immunol (1974) **113**, 316-322.
13. Tanizaki Y, Sudo M, Kitani H, Araki H, Oki K, Tsuji M, Takahasi K and Kimura I: Eosinophilic leucocytes and arylsulfatase activity in bronchoalveolar lavage fluid of patients with bronchial asthma. Acta Med Okayama (1988) **42**, 227-230.

Received February 23, 1994; accepted November 22, 1994.